Methods and Compositions for the Treatment of Myocardial Conditions

Technical Field

The present invention relates to methods for the treatment of conditions characterised by abnormalities of myocardial cell ion levels, such as Na^+, K^+ and Ca^{2+} ions. The present invention also relates to methods of treatment of heart failure and myocardial hypertrophy. The invention also relates to compounds and compositions useful in the treatment of mammals, particularly humans, having a condition characterised by abnormalities of myocardial cell ion levels, such as Na^+, K^+ and Ca^{2+} ions, including heart failure and myocardial hypertrophy.

Background

There are a number of conditions affecting humans and other animals which are characterised by abnormalities of myocardial cell ion levels, such as Na^+, K^+ and Ca^{2+} ions. Such conditions include heart failure, myocardial hypertrophy and hypertrophic cardiomyopathy.

In the Western world the prevalence of symptomatic heart failure is approximately 2%. In the United States ~5 million people are treated for it and it is estimated that 50-60 million Americans (~20%) are at risk of developing heart failure (Gheorghiade *et al.*, 2003). This group includes people suffering from one or more of the leading causes for development of heart failure, *i.e.* coronary artery disease, diabetes, hypertension or valvular heart disease.

Heart failure (HF), or congestive heart failure, represents a complex clinical syndrome characterised by abnormalities of left ventricular function and neurohormonal regulation. In general terms it is a condition characterised by reduced pumping capacity of the heart, which compromises perfusion of the body, initially typically during exertion, and with worsening HF also at rest. The reduced pumping capacity can be due to reduced contractile force of the heart muscle, the myocardium (systolic failure), or due to compromised re-filling of the heart, *i.e.* compromised myocardial relaxation (diastolic failure). HF develops due to a wide spectrum of aetiological and pathophysiological factors and varies considerably in clinical presentation.

The symptoms generally seen in HF are exertional dyspnoea, chest discomfort, orthopnoea, reduced exercise capacity, fluid retention causing weight gain, anorexia and nausea, fatigue and weakness, and in advanced stages central nervous system symptoms,

cachexia and reduced longevity. However, even severely reduced pump function may be associated with none or only few symptoms.

The neurohormonal abnormalities characteristic of HF include raised serum- and tissue levels of angiotensin II, catecholamines, in particular norepinephrine (noradrenaline), aldosterone, endothelin and natriuretic peptides. Addressing these abnormalities has been the foundation for clinical trials of pharmacological treatment. Drugs that antagonise the effect of angiotensin II (receptor blockers) or prevent its synthesis, angiotensin converting enzyme (ACE) inhibitors, aldosterone antagonists and β-adrenoceptor blockers significantly reduce morbidity and mortality in HF. Trials of endothelin receptor blockers and of drugs designed to raise levels of natriuretic peptides have not shown benefit.

In myocardial hypertrophy the increase in ventricular mass is paralleled by myocyte hypertrophy, i.e. increased size of the heart cells. Clinically, myocardial hypertrophy is often diagnosed by electrocardiography or by echocardiography showing an increased ventricular wall thickness. However, if the heart is also dilated, hypertrophy may be present with normal or even thin ventricular walls. Hypertrophy most often develops as a compensatory mechanism that helps the heart to deal with an increase in workload, e.g. due to heart valve disease or arterial hypertension. Myocardial hypertrophy may also develop due to abnormalities in the genes encoding the contractile proteins in the myocyte (Bundgaard *et al.*, 1999). This disease entity is associated with considerable morbidity and a high mortality rate.

In a recent meta-analysis of the prognostic implications of myocardial hypertrophy, Vakili *et al.* (2001) reported that myocardial hypertrophy consistently predicts a high risk for morbidity as well as mortality, independently of examined covariates, with no clear difference in relation to race, presence or absence of hypertension or coronary disease, or between clinical and epidemiologic samples. These results clarify the strong relation between ventricular hypertrophy and adverse outcome and emphasize the clinical importance of its detection. A meta-analysis of the outcome of medical management of this condition concluded that regression of left ventricular hypertrophy during antihypertensive treatment is associated with a marked reduction in risk for subsequent cardiovascular disease (Verdecchia *et al*, 2003).

Excitation-contraction coupling in the heart

In response to the cardiac action potential Ca^{2+} ions enter the cell from the outside and "trigger" release of Ca^{2+} from the sarcoplasmic reticulum (SR). The Ca^{2+} interact with contractile proteins of the cell and initiate contraction of the heart.

Relaxation of the heart depends on removal of Ca^{2+}. This occurs by re-uptake of Ca^{2+} into the SR and by extrusion across the cell membrane. Extrusion of Ca^{2+} across the cell membrane occurs mainly *via* an exchange mechanism that transports Ca^{2+} out in exchange for Na^+ that is transported in.

Abnormalities in cellular handling of Ca^{2+} during excitation-contraction coupling are involved in the pathogenesis of HF and myocardial hypertrophy. Raised intracellular levels of Na^+ in HF and myocardial hypertrophy is associated with a decrease in the electrochemical driving force that extrudes Ca^{2+} in exchange for Na^+. The high Na^+ levels may also be responsible for abnormalities in contraction.

The "uphill" transport of Ca^{2+} ions out of cells, against their electrochemical gradient, depends on the energy stored in an oppositely directed electrochemical gradient for Na^+. The transmembrane Na^+ gradient, in turn, is maintained by the Na^+-K^+ pump, effectively the only export route for Na^+. The pump transports 3 Na^+ ions out of cells in exchange for 2 K^+ ions that are transported in.

The mortality in HF is extremely high and increases with age and co-morbidities. In a study of 38,000 patients with a first admission for HF one year mortality was 33.1% (Jong P *et al*, 2002). The overall 5-year mortality for all patients after the HF diagnosis is established is ~50%. Despite a marked progress in the pharmacological management of HF the 5-year mortality has only come down from 60-70% in the period 1950-1969 to 50-60% in the period 1990-1999 (Levy D, 2002). Morbidity and mortality are thus still very high in heart failure despite administration of presently available drugs with proven efficacy.

Similar to the situation with heart failure, whilst there are treatments available for other conditions characterised by abnormalities of cellular ion levels each of the available treatments suffers one or more disadvantages and would benefit from the availability of improved methods of treatment.

Accordingly, there is a need for improved methods for the treatment of conditions characterised by abnormalities of myocardial cell ion levels, in particular abnormalities of cellular Na^+, K^+ or Ca^{2+} ions. In a particular focus, there is a need for improved methods for the treatment of HF and myocardial hypertrophy, and for improved agents and treatment regimes for HF and myocardial hypertrophy.

Summary of the Invention

The present invention aims to provide improved methods for the treatment of conditions characterised by abnormalities of myocardial cell ion levels, in particular abnormalities of cellular Na^+, K^+ or Ca^{2+} ions and so to substantially alleviate deficiencies of current treatments for such conditions. In a particular embodiment the present invention aims to provide improved methods for the treatment of heart failure and myocardial hypertrophy and so to substantially alleviate deficiencies of current treatments.

The present invention is based on the surprising discovery by the inventors that a myocardial cell surface receptor, the β_3 adrenoceptor, activates the membrane Na^+-K^+ pump and is associated with extrusion of Na^+ from myocardial cells.

Accordingly, in a first embodiment of the present invention there is provided a method for the treatment of an individual having a condition characterised by abnormal myocardial cell Na^+, K^+ or Ca^{2+} ion levels, said method comprising administering a therapeutically effective amount of one or more β_3 adrenoceptor agonists to said individual.

In a specific aspect the condition is selected from the group consisting of heart failure and/or myocardial hypertrophy.

According to a second embodiment of the invention there is provided a method for the treatment of an individual suffering from or susceptable to heart failure or myocardial hypertrophy, said method comprising administering a therapeutically effective amount of one or more β_3 adrenoceptor agonists to said individual.

In a specific aspect of the invention the individual is an individual suffering from one or more clinical symptoms of heart failure or myocardial hypertrophy.

In a specific aspect of the invention the β_3 adrenoceptor agonist is selected from the β_3 adrenoceptor agonist groups arylethanolamines, aryloxypropanolamines, trimetoquinols.

In a specific aspect of the invention the β_3 adrenoceptor agonist may be selected from the group consisting of BRL37344, BRL 35135, BRL 26830, BRL 26830A, BRL 35113, ZD7114, ZD 2076, CGP12177, CGP 12177A, CGP-20712A, CL316243, ICI07114, ICI D 7114, ICI215001, ICI 201651, BRL35135A, BRL28410, N-5984, (R)-N-[4-[2-[[2-Hydroxy-2-(pyridin-3-yl)ethyl]amino]ethyl]phenyl]- 4-[4-(4-trifluoro-methylphenyl)thiazol-2-yl]benzenesulfonamide (L-796568), (R)-N-[4-[2-[[2-hydroxy-2-(3-pyridinyl)- ethyl]amino]ethyl]phenyl]-1-(4-octylthiazol-2-yl)-5-indolinesulfonamide (L-755507), L-770,644, L-766,892, L-757,793, L-796568, LY-377604, Ro 40-2148, Ro

16-8714, SB-220646, SB-226552, SB-251023, SB-262552, SR 58306, SR 58375, SR 58339, SR 58611, SR 58611A, SR 59119A, GR-265261-X, AD-9677 and beta 3 agonist series 2-(3-indolyl) alkylamino-1-1(3-chlorophenyl)ethanols.

In a specific aspect of the invention the β_3 adrenoceptor agonist further comprises β_1 antagonist activity.

In a specific aspect of the invention the β_3 adrenoceptor agonist further comprises β_2 antagonist activity.

In a specific aspect of the invention the method further comprises administering one or more β blockers to said individual.

In a specific aspect of the invention the β blocker is a β_1 adrenoceptor antagonist.

In a specific aspect of the invention the β blocker may be administered to said individual prior to, simultaneously with or subsequent to administration of the one or more β_3 adrenoceptor agonists.

In a specific aspect of the invention the method comprises at least partially stabilizing said individual prior to administration of said β_3 adrenoceptor agonist.

In a specific aspect of the invention the method comprises commencement of a β_3 adrenoceptor agonist as an initial pharmacological approach in the treatment.

In a specific aspect of the invention said stabilizing comprises treatment with one or more compounds selected from the group consisting of ACE-inhibitors, aldosterone antagonists and β adrenoceptor antagonists.

According to a third embodiment of the invention, there is provided a method for treatment of a condition characterised by abnormally high myocardial cell Na^+ ion level, said method comprising administration to an individual having said condition of a therapeutically effective amount of one or more β_3 adrenoceptor agonists.

In a specific aspect of the third embodiment of the invention the condition characterised by abnormally high cellular Na^+ is myocardial hypertrophy.

In a specific aspect of the third embodiment of the invention the condition characterised by abnormally high cellular Na^+ is heart failure.

According to a fourth embodiment of the invention there is provided use of one or more β_3 adrenoceptor agonists for the manufacture of a medicament for the treatment of an individual having a condition characterised by abnormal myocardial cell Na^+, K^+ or Ca^{2+} ion levels.

According to a fifth embodiment of the invention there is provided one or more β_3 adrenoceptor agonists for use in the treatment of an individual having a condition characterised by abnormal myocardial cell Na^+, K^+ or Ca^{2+} ion levels.

According to a sixth embodiment of the invention there is provided use of one or more β_3 adrenoceptor agonists in the manufacture of a medicament for the treatment of an individual suffering from or susceptable to heart failure or myocardial hypertrophy.

According to a seventh embodiment of the invention there is provided one or more β_3 adrenoceptor agonists for use in the treatment of an individual suffering from or susceptable to heart failure or myocardial hypertrophy.

According to an eighth embodiment of the invention there is provided a pharmaceutical composition for use in the treatment of an individual having a condition characterised by abnormal myocardial cell Na^+, K^+ or Ca^{2+} ion levels, the composition comprising one or more β_3 adrenoceptor agonists together with one or more pharmaceutically acceptable adjuvants, excipients and/or carriers.

According to a ninth embodiment of the invention there is provided a pharmaceutical composition for use in the treatment of an individual suffering from or susceptable to heart failure or myocardial hypertrophy, the composition comprising one or more β_3 adrenoceptor agonists together with one or more pharmaceutically acceptable adjuvants, excipients and/or carriers.

According to a tenth embodiment of the invention there is provided a pharmaceutical composition comprising one or more β_3 adrenoceptor agonists and one or more β_1 and/or β_2 adrenoceptor antagonists, together with one or more pharmaceutically acceptable adjuvants, excipients and/or carriers.

According to an eleventh embodiment of the invention there is provided a method for the extrusion of Na^+ from a myocardial cell or cells, the method comprising contacting said cell(s) with one or more β_3 adrenoceptor agonist(s). The method may comprise Na,K pump stimulation. In a specific aspect the method may be an *in vitro* or an *in vivo* method.

Definitions

The term "therapeutically effective amount" as used herein includes within its meaning a non-toxic but sufficient amount of a compound or composition for use in the invention to provide the desired therapeutic effect. The exact amount required will vary from subject to subject depending on factors such as the species being treated, the age and general condition of the subject, co-morbidities, the severity of the condition being treated, the particular agent being administered and the mode of administration and so forth. Thus, it is not possible to specify an exact "effective amount". However, for any

given case, an appropriate "effective amount" may be determined by one of ordinary skill in the art using only routine methods.

In the context of this specification, the general class of receptors known as "beta (β) adrenoceptors" is relevant. In this context "beta adrenoceptor" or "β adrenoceptor" is understood to have its standard meaning in the art which, generally stated, includes β_1 adrenoceptors, β_2 adrenoceptors, β_3 adrenoceptors, which are receptors for circulating catecholamines (adrenaline and noradrenaline and derivatives thereof).

Similarly, the term "β_3 adrenoceptors" will be understood to have its usual meaning in the art which, generally stated, includes receptors known by alternative terminologies including atypical β adrenoceptor, β_3 adrenoceptors receptor, β_3 adrenergic receptor, and β_3 adrenaline receptor.

In the context of this specification, an agonist is any substance, agent or drug that stimulates physiological activity of a cell receptor that is normally capable of being stimulated by a naturally occurring or endogenous regulatory substance. It is to be understood that, in the context of this specification, the term "agonist" includes partial agonists in which the substance, agent or drug may be only partly as effective as an endogenous regulatory substance. It will be understood that, in the context of this specification, the term "agonist" includes any substance, agent or drug that acts directly on a receptor, including those which have affinity for a receptor, and any substance, agent or drug which may act indirectly on a receptor, such as through one or more intermediate substance(s) or pathways, to stimulate activity of the receptor.

In the context of this specification, the term "β_3 agonist" and "β_3 adrenoceptor agonist" includes within its scope any agent capable of activating a β_3 adrenoceptor, in particular any agent capable of activating a human β_3 adrenoceptor.

In the context of this specification, the term "selective", when used in the context of a β adrenoceptor agonist or β adrenoceptor antagonist, means that the agonist or antagonist has preferential, but not necessarily sole, activity toward one or more of the β_1, β_2, or β_3, adrenoceptors. For example, a β_3 adrenoceptor selective agonist has preferential stimulatory activity toward the β_3 adrenoceptor. For example, a β_1 adrenoceptor selective antagonist has preferential inhibitory effect toward the β_1 adrenoceptor.

In the context of this specification, the term "β blocker" means any substance, agent or drug that has antagonist or inhibitory activity toward a β_1 adrenoceptor and/or a β_2 adrenoceptor.

In the context of this specification, "heart failure" (HF) includes both chronic and acute heart failure. Thus, for example, it is expected that the method of the invention is

suitable for treatment of chronic heart failure and for treatment of acute heart failure. It will also be understood that, in the context of this specification the term "heart failure" includes symptomatic heart failure and asymptomatic heart failure, and therefore includes susceptability to acute or chronic heart failure. It will also be understood that, in the context of this specification, the term "heart failure" includes systolic heart failure and diastolic heart failure.

In the context of this specification, the term "patient" includes humans and individuals of any species of social, economic or research importance including but not limited to members of the genus ovine, bovine, equine, porcine, feline, canine, primates, rodents.

In the context of this specification, the term "combined with" and similar terms such as "in conjunction with" when used in relation to a therapeutic regime means that each of the drugs and other therapeutic agent(s), such as agonists and antagonists, is used in the treatment of an individual and that each of the drugs and other therapeutic agents in the "combined" therapeutic regime may be administered to the individual simultaneously with one or more of the other agents in the therapeutic regime, or may be administered to the individual at a different time to one or more of the other agents in the therapeutic regime. That is, the term "combined with" and similar terms such as "in conjunction with" when used in relation to a therapeutic regime may mean that any one or more of the drugs or other agents may be physically combined prior to administration to the patient, and it will be understood that the term also includes administration of the one or more drugs and other therapeutic agents as separate agents not in prior physical combination.

In the context of this specification, the term "comprising" means "including principally, but not necessarily solely". Furthermore, variations of the word "comprising", such as "comprise" and "comprises", have correspondingly varied meanings.

All references cited herein are incorporated by reference in their entirety.

Abbreviations

In the context of this specification, "HF" is an abbreviation for heart failure.

In the context of this specification, "SR" is an abbreviation for sarcoplasmic reticulum.

In the context of this specification, "CHF" is an abbreviation for congestive heart failure.

In the context of this specification, "LV" is an abbreviation for left ventricular.

In the context of this specification, "I_p" is an abbreviation for the measurable cellular transmembranous current generated by the Na,K-pump in experimental situations using a patch clamp technique.

In the context of this specification, "NMG.Cl" is an abbreviation for N-methyl-D-glucamine.

In the context of this specification, "ESPVR" is an abbreviation for left ventricular end systolic pressure-volume relationship.

In the context of this specification, "PKA" is an abbreviation for cAMP-activated protein kinase.

In the context of this specification, "H-89" is an abbreviation for (N-[2-(p-Bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide dihydrochloride.

Figure Legends

Figure 1 demonstrates the effects of the β_3 adrenoceptor agonist BRL 37344 on myocardial Na,K-pump-mediated current at agonist concentrations of 1 nM, 10 nM and 100 nM.

Figure 2 demonstrates the effects of the β_3 adrenoceptor agonist BRL 37344 on myocardial Na,K-pump-mediated current at an agonist concentration of 100 nM in the absence and presence of the β_1/β_2 blocker nadolol at 1 μM.

Figure 3 demonstrates the effects of the β_3 adrenoceptor agonist BRL 37344 on myocardial Na,K-pump-mediated current at an agonist concentration of 10 nM in the absence of extracellular Na^+ (NaCl was replaced by NMG.Cl).

Figure 4 demonstrates the effects of the naturally occurring catecholamine, noradrenaline (NA), on myocardial Na,K-pump-mediated current at an agonist concentration of 10 nM in the absence and presence of nadolol (1 μM; a β_1/β_2 blocker) and in the absence and presence of H-89 (0.5 μM; an inhibitor of PKA).

Figure 5 demonstrates the effects of selective activation of β_1/β_2 adrenergic-coupled intracellular messenger pathway, PKA, using the cAMP analogue 6-Bnz-cAMP (100 μM) in the absence and presence of H-89 (0.5 μM).

Figure 6 demonstrates the effects of BRL 37344 administration in the sheep heart failure model (Huang *et al.,* 2004), before and after induction of heart failure, using the left ventricular end systolic pressure-volume relationship (ESPVR) as an index of cardiac performance.

Detailed Description of the Invention and Best Mode

The invention will now be described in more detail, including, by way of illustration only, with respect to the examples which follow.

This invention is a completely new pharmacological approach to management of patients, especially human patients, with conditions characterised by abnormalities of myocardial cell ion levels, in particular abnormalities of cellular Na^+, K^+ or Ca^{2+} ions. In a particular embodiment, this invention provides a completely new pharmacological approach to the management of patients with heart failure (HF) or myocardial hypertrophy.

The invention is based on the inventors' surprising discovery that a myocardial cell surface receptor, the β_3 adrenoceptor, activates the membrane Na^+-K^+ pump to extrude Na^+ from cells. Since cardiac myocyte Na^+ is implicated in the pathophysiology of heart failure the discovery allows a rational design of a new pharmacological approach to treatment of HF using β_3 adrenoceptors activating drugs (β_3 agonists).

All cells, including cardiac myocytes, are characterised by a difference in electrical potential between the inside of the cell and the outside, separated by the cell membrane. The cell membrane in cardiac myocytes is called the sarcolemma. During the cardiac cycles the membrane potential undergoes cyclical changes with the inside transiently changing from a negative potential in the resting state to a positive potential. This change in membrane potential is called the cardiac action potential. Events during the cardiac action potential are crucial for myocyte contraction and hence pumping function of the heart. The interaction between the cardiac action potential and contraction is often referred to as "excitation-contraction coupling".

The change in membrane potential during the cardiac action potential activates specialised channels in the membrane to transiently become permeable to Ca^{2+} ions. The ions enter the cell from an area of high concentration on the outside to one of low concentration on the inside. The Ca^{2+} that enters serves as a "trigger" to release Ca^{2+} stored in intracellular structures called the sarcoplasmic reticulum (SR). The Ca^{2+} that enters *via* channels or is released from the SR combines to interact with contractile proteins of the cell and initiate contraction of the heart.

Relaxation of the heart is just as important as contraction. Relaxation depends on removal of Ca^{2+}. This occurs by an energy-dependent re-uptake of Ca^{2+} into the SR and by its extrusion from the inside of the cell to the surrounding outside milieu, across the cell membrane. Extrusion of Ca^{2+} across the cell membrane occurs *via* an energy-dependent Ca^{2+} pump and via a Na^+-Ca^{2+} exchange mechanism that transports Ca^{2+} out in

exchange for Na^+ that is transported in. In most mammalian species, including man, the Na^+-Ca^{2+} exchange is quantitatively the most important pathway for the extrusion of Ca^{2+}.

Abnormalities in cellular handling of Ca^{2+} during excitation-contraction coupling are pivotal in the pathogenesis of HF. Typically, there is a delay in the decline in intracellular Ca^{2+} after the transient increase that initiates contraction. Many mechanisms have been proposed, including abnormalities in the function and/or abundance of the membrane proteins that mediate re-uptake of Ca^{2+} into the SR or exchange with Na^+ across the sarcolemmal membrane.

It has recently been demonstrated that raised intracellular levels of Na^+ (and hence a decrease in the electrochemical driving force that extrudes Ca^{2+} in exchange for Na^+) is a pivotal abnormality in HF. This applies to both animals with experimentally induced HF and humans with the clinical condition. The raised intracellular Na^+ levels may be a major cause of abnormal cellular Ca^{2+} handling because of the role of the transmembrane Na^+ gradient in cellular extrusion of Ca^{2+} *via* Na^+-Ca^{2+} exchange.

Thus the high Na^+ levels may be responsible for abnormalities in contraction and they may activate gene programming present earlier in life and contribute to abnormal cardiac myocyte growth and adverse cardiac remodelling. While the presence of high intracellular Na^+ levels in HF and myocardial hypertophy are now widely accepted, the mechanism for the increase is uncertain. Enhanced Na^+ influx as well as diminished Na^+ efflux have been proposed.

The role of myocardial Na^+ and Na^+, K^+-pumps in HF

As described above, the "uphill" transport of Ca^{2+} ions out of cells, against their electrochemical gradient, depends on the energy stored in an oppositely directed electrochemical gradient for Na^+ (high concentration on the outside, low concentration on the inside). The transmembrane Na^+ gradient, in turn, is maintained by the Na^+-K^+ pump, effectively the only export route for Na^+. The pump transports 3 Na^+ ions out of cells in exchange for 2 K^+ ions that are transported in. It is an energy-dependent process that depends on hydrolysis of ATP for its operation, hence it is often referred to as the Na^+-K^+-ATPase.

The role of the Na^+-K^+ pump in excitation-contraction coupling has been recognised for approximately 45 years. The best known example of its involvement relates to the effect of cardiac glycosides, *e.g.* digoxin. This group of drugs are highly specific inhibitors of the Na^+-K^+ pump. Exposure of normal cardiac tissue to cardiac glycosides causes an increase in intracellular Na^+, an increase in Ca^{2+} and hence enhanced

contractility. This scheme has formed the foundation for use of cardiac glycosides in the treatment of HF. However, only modest glycoside-induced increases in intracellular Na^+ enhance contraction, and an increase in intracellular Na^+ of a few mM beyond "normal" levels start having an adverse effect on contraction. As described above intracellular Na^+ is already raised in HF and further increases are not likely to be beneficial. In agreement with this, although treatment with cardiac glycosides may be beneficial in terms of temporary symptomatic improvement, it does not improve survival in HF and even has an adverse effect on mortality in some subsets of patients.

The present invention is directed to measures with the potential to cause Na^+-K^+ pump *stimulation* in HF and other conditions. Prior to the present invention the use of β_3-adrenoceptor agonists had not been explored as a potential Na^+-K^+ pump activator.

β_3 adrenoceptors

β_3 adrenoceptors are expressed in many tissues, including the heart, and are present in many species including humans. Prior to the present invention the primary clinical and pharmacological interest in β_3 adrenoceptors has been related to a potential role of β_3 adrenoceptors agonists ("activators") in the treatment of obesity and type II diabetes mellitus, based on findings in rodents that β_3 adrenoceptors agonists induce an increase in metabolic rate, a lipolytic effect and an increased insulin sensitivity.

Studies on isolated cardiac tissue, including tissue from humans, have established that β_3 adrenoceptor agonists mediates a negative inotropic effect, *i.e.* a decrease in contractility. This is in marked contrast to β_1 and β_2 receptors agonists that mediate positive inotropy, *i.e.* an increase in contractility. The mechanism for the negative inotropic effect of β_3-adrenoceptors has not been firmly established, but has been suggested to be mediated by calcium channel blockage.

β_3 adrenoceptors are upregulated with the development of HF. It has been suggested that β_3 adrenoceptors have adverse effects on the progression of HF. Gauthier *et al.* (1998) thus suggested that catecholamine-activated β_3 adrenoceptor-mediated pathways may lead to myocardial dysfunction. It has also been proposed that the negative inotropic effect mediated by β_3 adrenoceptors is maladaptive and aggravates systolic dysfunction, particularly with severe HF (Moniotte *et al.*, 2001). Cheng *et al.* (2001) suggest that the β_3 adrenoceptor contributes to progression of cardiac dysfunction in HF. Morimoto *et al* (2004) reported that blocking β_3 adrenoceptor in a model of cardiomyocyte dysfunction in heart failure improved left ventricular (LV) contraction and relaxation and that stimulation of β_3 adrenoceptor by the high levels of catecholamines in

HF contributes to the impaired LV contraction and relaxation. Morimoto *et al* propose that the clinical implications of their results include that β_3 adrenoceptor blockade may provide a pharmacological method of inotropic support for the failing heart and that chronic β_3 adrenoceptor blockade might have favourable effects in HF.

In view of the role of the Na^+-K^+ pump in cardiac contraction (pump inhibition expected to have positive inotropic effect, pump stimulation expected to have a negative inotropic effect), the present inventors tested the hypothesis that the β_3 adrenoceptor is linked to activation of the Na^+-K^+ pump. The inventors describe herein that, surprisingly, the β_3 adrenoceptor can mediate an increase in the activity of sarcolemmal Na^+-K^+ pump in isolated cardiac myocytes. This is the first demonstration of an effect of β_3 adrenoceptors on regulation of myocardial Na^+-K^+ pump activity.

This β_3 adrenoceptor-mediated stimulation of the Na,K-pump will increase the cellular efflux of Na^+. Since raised levels of cardiac myocyte Na^+ is a hallmark of heart failure and myocardial hypertrophy such stimulation of Na^+ efflux may be beneficial. In support of this proposal, the well documented effective treatments with ACE-inhibitors and aldosterone antagonists are associated with Na-K pump stimulation in cardiac myocytes (Hool, LC *et al.*, *Am J Physiol* 271:C172-180, 1996, Mihailidou AS *et al.*, *Circ Res* 86: 37-42, 2000). The effects of these groups of drugs have the property in common of counteracting inhibitory effects of the naturally occurring hormones angiotensin II and aldosterone on the Na-K pump. This invention describes a new receptor-mediated method of directly stimulating the pump rather than merely preventing inhibition. The effects of the method may be additive to that of the already existing treatments. Initially a decrease in intracellular Na^+ levels may cause some deterioration in myocardial contractility. However, any possible initial deterioration should be followed by improvement in parallel with a gradual reduction in intracellular Na^+ concentration. Thus, the discovery of stimulation of the myocardial Na,K-pump by beta 3 adrenoceptor agonists provides a completely new pharmacological approach to the management of patients with heart failure or myocardial hypertrophy using beta 3 adrenoceptor activating drugs (beta 3 adrenoceptor agonists).

Accordingly, the present invention provides for the use of one or more β_3 adrenoceptor agonists in the treatment of HF or myocardial hypertrophy.

β_3 adrenoceptor agonists

β_3 adrenoceptor agonists are known in the art and include, but are not limited to, the β_3 adrenoceptor agonist groups arylethanolamines, aryloxypropanolamines,

trimetoquinols including, but not limited to, BRL37344, BRL 35135, BRL 26830, BRL 26830A, BRL 35113, ZD7114, ZD2076, CGP12177, CGP 12177A, CGP-20712A, CL316243, ICI07114, ICI D 7114, ICI215001, ICI 201651, BRL35135A, BRL28410, N-5984, (R)-N-[4-[2-[[2-Hydroxy-2-(pyridin-3-yl)ethyl]amino]ethyl]phenyl]- 4-[4-(4-trifluoro-methylphenyl)thiazol-2-yl]benzenesulfonamide (L-796568), (R)-N-[4-[2-[[2-hydroxy-2-(3-pyridinyl)- ethyl]amino]ethyl]phenyl]-1-(4-octylthiazol-2-yl)-5-indolinesulfonamide (L-755507), L-770,644, L-766,892, L-757,793, L-796568, LY-377604, Ro 40-2148, Ro 16-8714, SB-220646, SB-226552, SB-251023, SB-262552, SR 58306, SR 58375, SR 58339, SR 58611, SR 58611A, SR 59119A, GR-265261-X, AD-9677, 6-[2-(R)-[[2-(R)-(3-chlorophenyl)-2-hydroxyethyl]amino]propyl]-2,3-dihydro-1, 4-benzodioxine-2-(R)-carboxylic acid (Yanagisawa *et al* 2000), salbutamol, isoproterenol, tetradydroisoquinoline compounds as described in US Patent No. 6,596,734 to Feller and Miller entitled "Tetradydroisoquinoline compounds for use as β_3 adrenoceptor agonists" and other β_3 adrenoceptor agonists known in the art, for example as described in US Patent No. 6,566,377 to Day and Lafontaine entitled "β_3 adrenergic receptor agonists and uses thereof", US Patent No. 6,696,486 to Bahl entitled "Medical use for atypical β-adrenoceptor agonists", US Patent No. 6,593,341 to Feller and Miller entitled "Beta 3-adrenoceptor agonists, agonist compositions and methods of making and using the same", International Patent Application No. PCT/US00/33222 entitled "Beta-3 adrenoceptor agonists" published as WO 01/42217, and agonists described in Harada *et al* (*Bioorg. Med. Chem. Lett.* 2003, 13(7):1301-1305) entitled "Novel and potent human and rat beta3-adrenergic receptor agonists containing substituted 3-indolylalkylamines", such as those of the series 2-(3-indolyl) alkylamino-1-(3-chlorophenyl)ethanols, for example AJ9677, and agonists included in *The Merck Index*, (13th Edition, Merck & Co., Whitehouse Station, N.J., USA). Additionally, β3 agonists described in Weyer, C., *et al.*, (*Diabetes Metab.* 1999, 25:11), Souza, J., *et al.*, (*Curr. Pharm. Des.* 2001, 7:1433), Weber, A. (*Annu. Rep. Med. Chem.* 1998, 33:193), Cantello, B. and Smith, S. (*Drugs Future* 1991, 16:797), Bloom, J. and Claus, T. (*Drugs Future* 1994, 19:23), Cecchi, R., *et al.*, (*Eur. J. Med. Chem.* 1994, 29:259), are also contemplated in the invention.

It will be appreciated that the method of the invention contemplates the use of any suitable β_3 adrenoceptor agonist and that specific groups and compounds are stated herein by way of exemplification and not by way of limitation.

It will be appreciated that reference to the agents includes all salt (acid or base salt) and hydrates, polymorphs, etc, forms of those agents.

The β_3 adrenoceptor agonist may be selective, including, but not limited to, BRL37344, CGP12177, CL316243, and selective compounds disclosed in US Patent No. 6,686,372 to Crowell *et al* entitled "Selective β_3 adrenergic agonists".

Also known in the art are compounds having β_3 adrenoceptor agonist activity and β_1 and/or β_2 adrenoceptor antagonist activity including, but not limited to, CGP12177. The present invention contemplates the use of such compounds in the treatment of HF or myocardial hypertrophy.

The use of β-adrenoceptor blockade in HF (through the use of antagonists of β_1 and/or β_2 adrenoceptor) is already firmly established as beneficial in the treatment of HF. As described herein, the present invention provides for the use of a β_3 adrenoceptor agonist in the treatment of HF or myocardial hypertrophy. It is also expected that a combined therapeutic regime, comprising use of a β_3 adrenoceptor agonist in conjunction with one or more β_1- and or β_2-adrenoceptor antagonists may provide improved therapeutic outcomes.

Accordingly, the present invention also provides a method for the treatment of HF or myocardial hypertrophy wherein one or more β_3 adrenoceptor agonists is administered to an individual in conjunction with one or more β_1- and or β_2-adrenoceptor antagonists. β-adrenoceptor antagonists in this aspect of the invention may be those with an established role in the treatment of HF.

β_1- and or β_2-adrenoceptor antagonists

Compounds and agents having β_1- and or β_2-adrenoceptor antagonist activity are known in the art and include, but are not limited to, alprenolol, amosulalol, atenolol, betaxolol, bethanidine, bevantolol, bisoprolol, bopindolol, bufuralol, bunitrolol, bupranolol, butafilolol, carazolol, carteolol, carvedilol, celiprolol, cetamolol, dilevalol, epanolol, indenolol, labetalol, mepindolol, metipranolol, metoprolol, moprolol, nadolol, nebivolol, nipradilol, oxprenolol, penbutolol, pindolol, pronethalol, propranolol, sotalol, sulfinalol, talinolol, tertatolol, tilisolol, timolol, toliprolol, and the like. β-adrenoceptor antagonists are also described in US Patent No. 4,678,786 to Roe *et al* entitled "Pharmaceutical compositions having β-adrenoceptor antagonist activity employing pyridazinone derivatives". β-adrenoceptor antagonists are described more fully in the literature, such as in Goodman and Gilman, *The Pharmacological Basis of Therapeutics* (9th Edition), McGraw-Hill, 1995; and in *The Merck Index*, (13th Edition), Merck & Co., Whitehouse Station, N.J., USA, 2004.

The β_1- and or β_2-adrenoceptor antagonist may be any relatively non-selective β-adrenoceptor antagonist including, but not limited to, alprenolol, oxprenolol, sotalol and timolol. The β_1- and or β_2-adrenoceptor antagonist may be a selective β_1-adrenoceptor antagonist, including, but not limited to, nebivolol, atenolol, bisopronol, betaxolol, metoprolol, practolol and CGP20712A. The β-adrenoceptor antagonist may be a selective β_2-adrenoceptor antagonist, including, but not limited to, ICI118551.

The β_1- and or β_2-adrenoceptor antagonist, at therapeutic amounts may have relatively low, or no, affinity for β_3-adrenoceptor and so leave the β_3-adrenoceptor relatively unblocked. Such an antagonist includes, but is not limited to, metoprolol.

It will be appreciated that the method of the invention contemplates the use of any suitable agent having β_1- and or β_2-adrenoceptor antagonist activity and that specific groups and compounds are stated herein by way of exemplification and not by way of limitation.

It will be appreciated that reference to the agents includes all salt (acid or base salt) and hydrates, polymorphs, etc, forms of those agents.

Centrally acting sympatolytic agents

Also known in the art is the use of centrally acting sympatolytic agents in the treatment of HF. It is expected that combined use of β_3 adrenoceptor agonists with a centrally acting sympatolytic agent may also provide improved therapeutic outcome for the treatment of HF.

Accordingly, the present invention also provides a method for the treatment of HF or myocardial hypertrophy wherein one or more β_3 adrenoceptor agonists is administered to an individual in combination therapy with one or more centrally acting sympatolytic agents. Centrally-acting sympatolytic agents are known in the art and include, but are not limited to, those listed in *The Merck Index*, (13^{th} Edition), Merck & Co., Whitehouse Station, N.J., USA, 2004.

Treatment regime

The most appropriate treatment regime for any particular patient may be determined by the treating physician and will depend upon a variety of factors including: the disorder being treated and the severity of the disorder; activity of the compound or agent employed; the composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration; the route of administration; the rate of sequestration of the agent or compound; the duration of the treatment; drugs used in

combination or coincidental with the treatment, together with other related factors well known in medicine.

Based on theoretical expectations, the present inventors envisage that a clinically significant negative inotropic effect may be experienced if β_3 adrenoceptor agonists were to be given to patients with HF. As such, a patient's condition may transiently deteriorate before their condition improves, as is the case with commencement of treatment with β-adrenoceptor blockade and ACE inhibitors in HF.

In the animal heart failure model exemplified herein (see Example 6) no such initial deterioration was observed.

Accordingly, in the method of the invention and, where desirable to reduce actual or anticipated transient deterioration of the condition of the patient, treatment with one or more β_3 adrenoceptor agonists may be used in a patient already stabilised on conventional treatment such as with ACE-inhibitors, including but not limited to captopril, enalapril and lisonopril, aldosterone antagonists and β_1- and or β_2-adrenoceptor antagonists. Furthermore, the clinical practice of "starting low and going slow" of antagonist treatment may also be followed for β_3 adrenoceptor agonist treatment.

The start low go slow principle means to start at such a low dose that adverse effects are very unlikely, but – on the other hand – no significant beneficial effects are expected either. After a period, which may be days or weeks, when no or only minor adverse effects have been observed a minor up-titration of the dose is performed. This scheme is continued until adverse effects are seen, for example ortostatic hypotension during beta-blocker up-titration, or until a recommended target dose is reached. It may take weeks to months from commencement until target dose is reached.

ACE-inhibitor, beta-blocker and aldosterone antagonist, such as spironolactone and eplerenone, are three classes of drugs proven to reduce symptoms and improve survival in patients with HF. As a further example of a treatment regime, current pharmacological management of HF includes the "add-on" principle. In such a regime, the patient may start treatment with an ACE inhibitor which may be titrated up to a certain level. Then treatment with a beta-blocker may be commenced (added-on) and titrated up to a certain level and then treatment with spironolactone is commenced. Conditions related to treatment incorporating the "add-on" principle are also included in a treatment regime using one or more β_3 adrenoceptor agonists.

Thus, in one aspect of the present invention, the administration of one or more β_3 adrenoceptor agonists may be as an "add-on", in which a patient may be treated with a drug from each of the three classes ACE-inhibitor, beta-blocker and aldosterone

antagonist, and before, during or after that treatment, treatment with one or more β_3 adrenoceptor agonists may be commenced, preferably using the start low go slow principle. Consequently, it will be appreciated that in this context the term "add-on" refers to an additional therapeutic integer (the β_3 adrenoceptor agonist); it does not mean that the β_3 adrenoceptor agonist must be added as the last drug. The order and composition of the specific drugs and drug classes in the combination therapy may be determined by the skilled addessee, and may include, for example where the therapeutic regime involves the administration of four drug classes, the β_3 adrenoceptor agonist may be the first, second, third, or fourth drug to be administered.

As a further example of a treatment regime of the method of the invention, the condition of a patient may be at least partially stabilized prior to administration of the one or more β_3 adrenoceptor agonists. Furthermore, the condition of a patient may be at least partially stabilized prior to commencement of a method of the invention. Either of such treatment regimes may be referred to as first stabilizing a patient.

First stabilising a patient includes, for example, where treatment with ACE-inhibitors and beta-blockers is not commenced in HF when the patient's cardiac function is severely compromised, typically with a low blood pressure and a low cardiac output. A reason for postponing commencement of the therapy is that these drugs may initially deteriorate the condition by inducing further reductions in blood pressure or cardiac output. On this basis the patient's condition is first stabilised, for example by optimisation of intravascular fluid volume (euvolemia), over a period of, for example, a few days before the "initial minor deterioration" is expected to be tolerated.

Conditions related to commencement of treatment with β_3 adrenoceptor agonists after first stabilizing a patient may be similar to the situation for ACE-inhibitor and beta-blocker treatment.

As a further example of a treatment regime, a β_3 adrenoceptor agonist may be the first drug to be adminstered in the treatment of heart failure without any prior medication or stabilisation. For example, commencement of β_3 adrenoceptor agonist treatment in a patient with only, or mainly, diastolic cardiac dysfunction or in patients with myocardial hypertrophy without significantly reduced cardiac systolic function is not expected to induce any initial side effects.

The compounds and agents proposed for the present invention may be administered as compositions either therapeutically or preventively. In a therapeutic application, compositions are administered, for example, to a patient already having heart failure whether symptomatic or not, in an amount sufficient to effectively treat the patient. In a

preventive application, compositions are administered to, for example, a patient having myocardial hypertrophy in an amount sufficient to effectively treat the patient thereby at least slowing or preventing disease progression. The composition should thus provide a quantity of the compound or agent sufficient to effectively treat the patient.

The therapeutically effective dose level for any particular patient will depend upon a variety of factors including: the disorder being treated and the severity of the disorder; activity of the compound or agent employed; the composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration; the route of administration; the rate of sequestration of the agent or compound; the duration of the treatment; drugs used in combination or coincidental with the treatment, together with other related factors well known in medicine.

One skilled in the art would be able, by routine experimentation, to determine an effective, non-toxic amount of the β_3 adrenoceptor agonist, and other agents where appropriate, which would be required to treat the condition.

Generally, an effective dosage is expected to be in the range of about 0.00001mg to about 1000mg per kg body weight per 24 hours; typically in the range of about 0.0001mg to about 1000mg per kg body weight per 24 hours; typically, about 0.001mg to about 750mg per kg body weight per 24 hours; about 0.01mg to about 500mg per kg body weight per 24 hours; about 0.1mg to about 500mg per kg body weight per 24 hours; about 0.1mg to about 250mg per kg body weight per 24 hours; about 1.0mg to about 250mg per kg body weight per 24 hours. More typically, an effective dose range is expected to be in the range about 1.0mg to about 200mg per kg body weight per 24 hours; about 1.0mg to about 100mg per kg body weight per 24 hours; about 1.0mg to about 50mg per kg body weight per 24 hours; about 1.0mg to about 25mg per kg body weight per 24 hours; about 5.0mg to about 50mg per kg body weight per 24 hours; about 5.0mg to about 20mg per kg body weight per 24 hours; about 5.0mg to about 15mg per kg body weight per 24 hours.

Alternatively, an effective dosage may be up to about 500mg/m^2. Generally, an effective dosage is expected to be in the range of about 25 to about 500mg/m^2, preferably about 25 to about 350mg/m^2, more preferably about 25 to about 300mg/m^2, still more preferably about 25 to about 250mg/m^2, even more preferably about 50 to about 250mg/m^2, and still even more preferably about 75 to about 150mg/m^2.

Typically, in therapeutic applications, the treatment would be for the duration of the disease state.

Further, it will be apparent to one of ordinary skill in the art that the optimal quantity and spacing of individual dosages and, where combination therapy is used, optimal quantity and spacing of administration of the various agents of the combination therapy, will be determined by the nature and extent of the disease state being treated, the form, route and site of administration, and the nature of the particular individual being treated. Also, such optimum conditions can be determined by conventional techniques.

It will also be apparent to one of ordinary skill in the art that the optimal course of treatment, such as, the number of doses of the composition or compositions given per day for a defined number of days, can be ascertained by those skilled in the art using conventional course of treatment determination tests.

Pharmaceutical compositions

In general, suitable compositions may be prepared according to methods which are known to those of ordinary skill in the art and accordingly may include a pharmaceutically acceptable carrier, diluent, excipient and/or adjuvant.

These compositions can be administered by standard routes. In general, the compositions may be administered by the parenteral (e.g., intravenous, intraspinal, subcutaneous or intramuscular), oral or topical route. Preferably administration is by the parenteral or oral route. More preferably administration is by the oral route.

The carriers, diluents, excipients and adjuvants must be "acceptable" in terms of being compatible with the other ingredients of the composition, and not deleterious to the recipient thereof.

Examples of pharmaceutically acceptable carriers or diluents are demineralised or distilled water; saline solution; vegetable based oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oils, arachis oil or coconut oil; silicone oils, including polysiloxanes, such as methyl polysiloxane, phenyl polysiloxane and methylphenyl polysolpoxane; volatile silicones; mineral oils such as liquid paraffin, soft paraffin or squalane; cellulose derivatives such as methyl cellulose, ethyl cellulose, carboxymethylcellulose, sodium carboxymethylcellulose or hydroxypropylmethylcellulose; lower alkanols, for example ethanol or iso-propanol; lower aralkanols; lower polyalkylene glycols or lower alkylene glycols, for example polyethylene glycol, polypropylene glycol, ethylene glycol, propylene glycol, 1,3-butylene glycol or glycerin; fatty acid esters such as isopropyl palmitate, isopropyl myristate or ethyl oleate; polyvinylpyrridone; agar; carrageenan; gum tragacanth or gum

acacia, and petroleum jelly. Typically, the carrier or carriers will form from 10% to 99.9% by weight of the compositions.

The composition may include agents which increase the bioavailability or therapeutic duration of the active compound or compounds.

The compositions of the invention may be in a form suitable for administration by injection, in the form of a formulation suitable for oral ingestion (such as capsules, tablets, caplets, elixirs, for example), in the form of an ointment, cream or lotion suitable for topical administration, in a form suitable for delivery as an eye drop, in an aerosol form suitable for administration by inhalation, such as by intranasal inhalation or oral inhalation, in a form suitable for parenteral administration, that is, subcutaneous, intramuscular or intravenous injection.

For administration as an injectable solution or suspension, non-toxic parenterally acceptable diluents or carriers can include, Ringer's solution, isotonic saline, phosphate buffered saline, ethanol and 1,2 propylene glycol.

Some examples of suitable carriers, diluents, excipients and adjuvants for oral use include peanut oil, liquid paraffin, sodium carboxymethylcellulose, methylcellulose, sodium alginate, gum acacia, gum tragacanth, dextrose, sucrose, sorbitol, mannitol, gelatine and lecithin. In addition these oral formulations may contain suitable flavouring and colourings agents. When used in capsule form the capsules may be coated with compounds such as glyceryl monostearate or glyceryl distearate which delay disintegration.

Adjuvants typically include emollients, emulsifiers, thickening agents, preservatives, bactericides and buffering agents.

Solid forms for oral administration may contain binders acceptable in human and veterinary pharmaceutical practice, sweeteners, disintegrating agents, diluents, flavourings, coating agents, preservatives, lubricants and/or time delay agents. Suitable binders include gum acacia, gelatine, corn starch, gum tragacanth, sodium alginate, carboxymethylcellulose or polyethylene glycol. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, guar gum, xanthan gum, bentonite, alginic acid or agar. Suitable diluents include lactose, sorbitol, mannitol, dextrose, kaolin, cellulose, calcium carbonate, calcium silicate or dicalcium phosphate. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid

and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

Liquid forms for oral administration may contain, in addition to the above agents, a liquid carrier. Suitable liquid carriers include water, oils such as olive oil, peanut oil, sesame oil, sunflower oil, safflower oil, arachis oil, coconut oil, liquid paraffin, ethylene glycol, propylene glycol, polyethylene glycol, ethanol, propanol, isopropanol, glycerol, fatty alcohols, triglycerides or mixtures thereof.

Suspensions for oral administration may further comprise dispersing agents and/or suspending agents. Suitable suspending agents include sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethyl-cellulose, poly-vinyl-pyrrolidone, sodium alginate or acetyl alcohol. Suitable dispersing agents include lecithin, polyoxyethylene esters of fatty acids such as stearic acid, polyoxyethylene sorbitol mono- or di-oleate, -stearate or -laurate, polyoxyethylene sorbitan mono- or di-oleate, -stearate or -laurate and the like.

The emulsions for oral administration may further comprise one or more emulsifying agents. Suitable emulsifying agents include dispersing agents as exemplified above or natural gums such as guar gum, gum acacia or gum tragacanth.

Methods for preparing parenterally administrable compositions are apparent to those skilled in the art, and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pa., hereby incorporated by reference herein.

The topical formulations of the present invention, comprise an active ingredient together with one or more acceptable carriers, and optionally any other therapeutic ingredients. Formulations suitable for topical administration include liquid or semi-liquid preparations suitable for penetration through the skin to the site of where treatment is required, such as liniments, lotions, creams, ointments or pastes, and drops suitable for administration to the eye, ear or nose. Formulations suitable for topical administration may be provided as a transdermal patch.

Drops according to the present invention may comprise sterile aqueous or oily solutions or suspensions. These may be prepared by dissolving the active ingredient in an aqueous solution of a bactericidal and/or fungicidal agent and/or any other suitable preservative, and optionally including a surface active agent. The resulting solution may

then be clarified by filtration, transferred to a suitable container and sterilised. Sterilisation may be achieved by: autoclaving or maintaining at 90°C-100°C for half an hour, or by filtration, followed by transfer to a container by an aseptic technique. Examples of bactericidal and fungicidal agents suitable for inclusion in the drops are phenylmercuric nitrate or acetate (0.002%), benzalkonium chloride (0.01%) and chlorhexidine acetate (0.01%). Suitable solvents for the preparation of an oily solution include glycerol, diluted alcohol and propylene glycol.

Lotions according to the present invention include those suitable for application to the skin or eye. An eye lotion may comprise a sterile aqueous solution optionally containing a bactericide and may be prepared by methods similar to those described above in relation to the preparation of drops. Lotions or liniments for application to the skin may also include an agent to hasten drying and to cool the skin, such as an alcohol or acetone, and/or a moisturiser such as glycerol, or oil such as castor oil or arachis oil.

Creams, ointments or pastes according to the present invention are semi-solid formulations of the active ingredient for external application. They may be made by mixing the active ingredient in finely-divided or powdered form, alone or in solution or suspension in an aqueous or non-aqueous fluid, with a greasy or non-greasy basis. The basis may comprise hydrocarbons such as hard, soft or liquid paraffin, glycerol, beeswax, a metallic soap; a mucilage; an oil of natural origin such as almond, corn, arachis, castor or olive oil; wool fat or its derivatives, or a fatty acid such as stearic or oleic acid together with an alcohol such as propylene glycol or macrogols.

The composition may incorporate any suitable surfactant such as an anionic, cationic or non-ionic surfactant such as sorbitan esters or polyoxyethylene derivatives thereof. Suspending agents such as natural gums, cellulose derivatives or inorganic materials such as silicaceous silicas, and other ingredients such as lanolin, may also be included.

The compositions may also be administered in the form of liposomes. Liposomes are generally derived from phospholipids or other lipid substances, and are formed by mono- or multi-lamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolisable lipid capable of forming liposomes can be used. The compositions in liposome form may contain stabilisers, preservatives, excipients and the like. The preferred lipids are the phospholipids and the phosphatidyl cholines (lecithins), both natural and synthetic. Methods to form liposomes are known in the art, and in relation to this specific reference is made to: Prescott, Ed.,

Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (1976), p. 33 *et seq.*, the contents of which is incorporated herein by reference.

Examples

The examples are intended to serve to illustrate this invention and should not be construed as limiting the general nature of the disclosure of the description throughout this specification.

Figures 1, 2 and 3 below summarise experiments demonstrating how the β_3 adrenoceptor-selective agonist BRL 37344 stimulates the membrane Na^+-K^+ pump in isolated rabbit cardiac myocytes. The experiments suggest that pump stimulation is reduced when high concentrations of the drug is used and that this reversal of a pump stimulatory effect is likely to be due to activation of β_1 and/or β_2 adrenoceptors at high BRL 37344 concentrations. The experiments demonstrate that the BRL 37344-induced increase in pump activity is due to direct Na^+-K^+ pump stimulation rather than secondary to a BRL 37344-induced increase in transmembrane Na^+ influx and stimulation of the pump by a resulting increase in the intracellular Na^+ concentration.

Example 1

The whole-cell patch clamp technique (Hamill *et al*, 1981; Buhagier *et al*, 2004; Hansen *et al*, 2002) was used to examine the effect of β_3 adrenoceptor stimulation on the Na^+-K^+ pump in isolated cardiac myocytes.

With the whole-cell patch clamp technique myocytes are suspended in a tissue bath mounted on the stage of an inverted microscope. A fine glass pipette is attached to the cell membrane and gentle suction applied to rupture the membrane patch directly under the tip of the glass pipette. This allows the intracellular compartment of the myocyte to be perfused with solution contained in the patch pipette. It also allows access of drugs and compounds to the intracellular compartment. Control of the composition of the extracellular solution in the tissue bath is achievable. The technique also allows control of membrane voltage and measurement of total membrane current.

The Na^+-K^+ pump's $3Na^+$:$2K^+$ exchange ratio generates a current (I_p) that can be measured as the shift in holding current of the patch-clamped myocyte that is induced by pump blockade with 100 μM ouabain.

In the whole-cell configuration the bath was perfused with modified Tyrode's solution which contained (in mmol/L) NaCl 140; KCl 5.6; $CaCl_2$ 2.16; $MgCl_2$ 1; NaH_2PO_4 0.44; glucose 10 and Na-glutamate 9; N-2-hydroxyethyl piperazine-N'-2-

ethene-sulphonic acid (HEPES) 10. The solution was titrated to a pH of 7.40 ± 0.01 at 35°C with NaOH.

For measurement of I_p the system was switched to a superfusate that usually was identical to the modified Tyrode's solution except that it was nominally Ca^{2+}-free and contained 0.2 mmol/L $CdCl_2$ and 2 mmol/L $BaCl_2$.

Wide-tipped patch pipettes (4-5 μm) were filled with solutions containing (in mmol/L) HEPES 5; MgATP 2; ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid (EGTA) 5; KCl 70 mmol/L. Osmotic balance was maintained with 80 mmol/L tetramethylammonium chloride (TMA-Cl). The solutions were titrated to a pH of 7.05 ± 0.01 at 35°C with 1 mmol/L TMA-OH. I_p was identified at a holding potential of –40 mV as the shift in holding current induced by Na^+-K^+ pump blockade with 100 μmol/L ouabain 10-15 minutes after the whole-cell configuration had been established. I_p is reported normalized for membrane capacitance and hence cell size. Details of patch pipette characteristics and equipment and criteria for defining I_p have been reported previously (Buhagiar *et al*, 1999).

The effects of different concentrations of BRL 37344 (Sigma Aldrich) on Na^+-K^+ pump current (I_p) was determined. The Na concentration in the pipette solution was 10 mM, *i.e.* at a near-physiological level. Figure 1 shows that in comparison with normal controls, BRL 37344 significantly stimulates the Na,K-pump at all three concentration levels. At the highest level (100 nM BRL 37344) an apparent lower activity was observed in comparison with activity observed with 10 nM. This is likely to be due to unspecific binding of BRL 37344 to β_1 and/or β_2 adrenoceptors at higher concentrations. β_1 and β_2 adrenoceptors agonists are expected to inhibit the myocardial Na,K-pump. Mean, SEM and number of experiments (n) values are indicated.

Example 2

See the description of the whole-cell patch clamp technique described in Example 1.

This experiment examined if the apparent decrease in stimulation of the Na,K-pump with exposure to 100 nM BRL 37344 compared to the stimulation seen with 10 nM BRL 37344 might be caused by stimulation of β_1 and/or β_2 adrenoceptors by the higher BRL 37344 concentration.

The effect of 100 nM BRL 37344 in the superfusate in the absence and in the presence of the combined β_1 and β_2 adrenoceptor blocker nadolol was measured in myocytes.

Figure 2 shows that blockage of β_1 and β_2 adrenoceptors with nadolol in the presence of 100 nM BRL 37344 increased, although not quite significantly, the activity of the Na,K-pump as compared to the stimulation seen with 100 nM BRL 37344 alone.

This suggests that the attenuated Na,K-pump stimulation with the high BRL 37344 concentration (see Figure 1) was a result of BRL 37344 stimulation of the β_1 and/or β_2 adrenoceptors. These results also confirm that the pump stimulation observed in response to the β_3 adrenoceptor agonist at the lower concentrations of 1 nM and 10 nM was mediated by β_3 rather than β_1 and/or β_2 adrenoceptors and suggest that β_1 and/or β_2 adrenoceptor activation may reduce Na,K-pump activity. Mean, SEM and number of experiments (n) values are indicated.

Example 3

The purpose of this experiment was to investigate if the β_3 adrenoceptor agonist BRL 37344-induced stimulation of the Na,K-pump is caused by, or is secondary to, increased influx of Na^+ into the cell. Increased intracellular Na^+ stimulates the Na^+-K^+ pump. If the effect of β_3 adrenoceptor were secondary to an increase of Na^+ influx rather than primarily Na^+-K^+ pump stimulation, then β_3 adrenoceptor agonists would be expected to be harmful in heart disease.

The whole-cell patch clamp technique was similar to that described above except that extracellular Na^+ was eliminated (NaCl was replaced with N-methyl-D-glucamine (NMG.Cl)) to rule out any possible influx of Na^+ across the cell membrane.

Figure 3 shows that BRL 37344-induced stimulation of the Na,K-pump persisted in the absence of extracellular Na^+. This result is consistent with stimulation having been due to an intrinsic change in Na^+-K^+ pump function rather than due to BRL 37344-induced transsarcolemmal Na^+ influx and so is consistent with direct Na^+-K^+ pump stimulation rather than a secondary effect.

Example 4

In the Examples 1, 2, and 3 the effect of the synthetic selective β_3 adrenergic agonist BRL 37344 on the sarcolemmal Na^+-K^+ pump was examined. In this example the effect of the naturally occurring catecholamine, noradrenaline (NA) was examined. The whole-cell patch clamp technique was used as described in Example 1.

Isolated cardiac myocytes were exposed to 10 nM NA and I_p was measured. As shown in Figure 4, NA induced an increase in I_p. We next examined the effect of NA on I_p when β_1- and β_2 adrenergic receptors were blocked with nadolol. Myocytes were

exposed to 10 nM NA and to 1 μM nadolol. Figure 4 demonstrates that the NA-induced increase in I_p persisted in the presence of nadolol. This strongly suggests that the increase is not mediated by β_1/β_2 adrenergic receptors.

To obtain further support for this conclusion the effect of blocking the intracellular messenger pathway classically activated by β_1/β_2 adrenergic receptors was examined. Activation of the β_1/β_2 adrenergic receptors results in intracellular synthesis of cAMP and subsequent activation cAMP-activated protein kinase (*a.k.a.* PKA). Myocytes were patch clamped using a patch pipette solution that contained 0.5 μM H-89 (N-[2-(p-Bromocinnamylamino)ethyl]-5-isoquinolinesulfonamide dihydrochloride (Calbiochem), an inhibitor of PKA. H-89 at this concentration abolishes PKA-mediated Na^+-K^+ pump inhibition (William *et al.*, 2003). As shown in Figure 4, the NA-induced increase in I_p persisted with PKA blockade.

Example 5

In a further set of experiments the effect of selectively activating β_1/β_2 adrenergic receptor-coupled intracellular messenger pathways was investigated. Since it is very difficult to experimentally selectively activate β_1/β_2 adrenergic membrane receptors with extracellular compounds we chose to directly activate the intracellular pathway known to be activated by the receptors, ie the cAMP-activated protein kinase (PKA). The cAMP analog 6-Bnz-cAMP activates PKA.

100 μM of 6-Bnz-cAMP was included in the patch pipette solutions that perfused the intracellular compartment. The myocytes were exposed to NA-free control superfusate. As shown in Figure 5, the analogue 6-Bnz-cAMP included in the pipette solution induced a significant decrease in I_p. To ascertain that the 6-Bnz-cAMP-induced decrease in I_p was mediated by PKA we also measured I_p using pipette solutions that included 6-Bnz-cAMP as well as H-89. The latter was expected to inhibit 6-Bnz-cAMP-activated PKA. Results are included in Figure 5 and demonstrate that H-89 (0.5 μM) reversed the 6-Bnz-cAMP-induced decrease in I_p.

Taken together, the results presented here indicate that activation of β_1/β_2 adrenergic receptors cause Na^+-K^+ pump inhibition while activation of β_3 adrenergic receptors causes Na^+-K^+ pump stimulation. This provides an explanation for the already established efficacy of β_1/β_2 adrenergic receptor blockers in human heart failure, and it identifies the β_3 adrenergic receptor as an entirely new drug target. Stimulation of the receptor is expected to promote Na^+ export from Na^+-overloaded cells in heart failure, thereby providing a beneficial therapeutic effect.

The role of the β_3 adrenergic receptor is supported by the inability of β_1/β_2 blockade with nadolol to block pump stimulation, by the persistence of stimulation when intracellular messenger pathways classically activated by β_1/β_2 receptors are blocked and by the effect of the selective β_3 adrenergic-selective agonist BRL 37344 to induce stimulation of I_p.

Example 6

Effect of BRL 37344 on cardiac performance in sheep heart failure model

To examine the effect of acute activation of the β_3-receptor we used a model of ischaemic cardiomyopathy with severe heart failure induced by repeated coronary microembolisation in sheep (Huang, Y *et al.*, *Am J Physiol* 286: H2141-50, 2004). The model is stable and reflects the human condition well (Huang *et al*, 2004).

BRL 37344 was administered by intravenous infusion over a period of 8 min, starting with the lowest dose of the protocol. After an additional 5 min pressure-volume relationships were determined as described (Huang *et al.*, 2004). The procedure was then repeated in an identical fashion using a larger dose of BRL 37344. The left ventricular end systolic pressure-volume relationship (ESPVR) was used as an index of cardiac performance. ESPVR is relatively independent of confounding factors such as pre- and after-load and heart rate, and is widely accepted as a good index of left ventricular function.

Results are shown in Figure 6, both before and after induction of heart failure. The independent variable on the abscissa indicates the dose in microgram BRL 37344 per kg weight of the sheep. Note the logarithmic increment in dose. The mean of ESPVR measured in 3 different sheep before and after induction of severe heart failure is shown on the ordinate. A linear regression analysis of a logarithmic transformation of the dose of BRL 37344 vs. ESPVR indicated a trend towards a negative slope in normal sheep consistent with a dose-dependent deterioration in left ventricular function. In contrast, the slope was significantly positive after heart failure had been induced in the sheep indicating a dose-dependent improvement in left ventricular function with administration of BRL 37344. It is concluded that β_3 adrenergic receptors activation has acute beneficial effects in heart failure, which is consistent with the expectations of the inventors based on the results presented herein for *in vitro* stimulation of the Na^+-K^+ pump.

Summary

A change in the intracellular Na^+ concentration generally has opposite effects under normal physiological conditions and under conditions of heart failure. In the normal heart, an increase in the intracellular Na^+ concentration causes an increase in the intracellular Ca^{2+} concentration and hence an enhancement of contractility. However, only modest increases in intracellular Na^+ improve cardiac performance, and an increase of a few mM beyond "normal" has adverse effects. In heart failure, intracellular Na^+ is already raised, and further increases impair performance. The present inventors describe, for the first time herein, a method for directly activating the cell membrane pump that extrudes Na^+ from the cells. As supported by the examples herein, this is expected to reduce Na^+ towards normal in the Na^+ overloaded cells and hence enhance cardiac performance. The method to activate Na^+ extrusion from cells involves the use of one or more β_3 adrenoceptor agonists.

Any description of prior art documents herein, or statements herein derived from or based on those documents, is not an admission that the documents or derived statements are part of the common general knowledge of the relevant art in Australia or elsewhere.

While the invention has been described in the manner and detail as above, it will be appreciated by persons skilled in the art that numerous variations and/or modifications including various omissions, substitutions, and/or changes in form or detail may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

List of References

Bloom, J. and Claus, T. *Drugs Future* 1994, 19:23.

Buhagiar KA, Hansen PS, Gray DF, Mihailidou AS and Rasmussen HH. Angiotensin regulates the selectivity of the Na+-K+ pump for intracellular Na+. *Am J Physiol* 277: C461-C468, 1999.

Buhagiar KA, Hansen PS, Kong BY, Clarke RJ, Fernandes C and Rasmussen HH. Dietary cholesterol alters Na+/K+ selectivity at intracellular Na+/K+ pump sites in cardiac myocytes. *Am J Physiol Cell Physiol* 286: C398-C405, 2004.

Bundgaard H, Havndrup O, Andersen PS, Larsen LA, Brandt NJ, Vuust J, Kjeldsen K, Christiansen M. Familial hypertrophic cardiomyopathy associated with a novel missense mutation affecting the ATP-binding region of the cardiac beta-myosin heavy chain. *J Mol Cell Cardiol.* 1999, 31(4):745-50.

Cantello, B. and Smith, S., *Drugs Future* 1991, 16:797.

Cecchi, R., *et al., Eur. J. Med. Chem.* 1994, 29:259.

Cheng HJ, Zhang ZS, Onishi K, Ukai T, Sane DC and Cheng CP. Upregulation of functional beta(3)-adrenergic receptor in the failing canine myocardium. *Circ Res* 89: 599-606, 2001.

Gauthier C, Leblais V, Kobzik L, Trochu JN, Khandoudi N, Bril A, Balligand JL and Le Marec H. The negative inotropic effect of beta3-adrenoceptor stimulation is mediated by activation of a nitric oxide synthase pathway in human ventricle. *J Clin Invest* 102: 1377-1384, 1998.

Gheorghiade, M. *et al* 2003, Braunwauld, Heart Diseases, 6th Edition.

Goodman and Gilman, *The Pharmacological Basis of Therapeutics* (9th Edition), McGraw-Hill, 1995.

Hamill OP, Marty A, Neher E, Sakmann B and Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflugers Arch* 391: 85-100, 1981.

Hansen PS, Buhagiar KA, Kong BY, Clarke RJ, Gray DF and Rasmussen HH. Dependence of Na+-K+ pump current-voltage relationship on intracellular Na+, K+, and Cs+ in rabbit cardiac myocytes. *Am J Physiol Cell Physiol* 283: C1511-C1521, 2002.

Harada, H *et al*, Novel and potent human and rat β_3-Adrenergic receptor agonists containing substituted 3-indolylalkylamines, *Bioorganic & Medicinal Chemistry Letters* 13 (2003): 1301-1305..

Hool, LC *et al., Am J Physiol* 271:C172-180, 1996.

Huang, Y., *et al.*, Remodeling of the chronic severely failing ischemic sheep heart after coronary microembolization: functional, energetic, structural, and cellular responses. *Am J Physiol Heart Circ Physiol* 286: H2141-50, 2004.

Jong P, Vowinckel E, Liu PP, Gong Y and Tu JV. Prognosis and determinants of survival in patients newly hospitalized for heart failure: a population-based study. *Arch Intern Med* 162: 1689-1694, 2002.

Levy D, Kenchaiah S, Larson MG, Benjamin EJ, Kupka MJ, Ho KK, Murabito JM and Vasan RS. Long-term trends in the incidence of and survival with heart failure. *N Engl J Med* 347: 1397-1402, 2002.

Mihailidou AS *et al.*, *Circ Res* 86: 37-42, 2000.

Moniotte S, Kobzik L, Feron O, Trochu JN, Gauthier C and Balligand JL. Upregulation of beta(3)-adrenoceptors and altered contractile response to inotropic amines in human failing myocardium. *Circulation* 103: 1649-1655, 2001.

Morimoto A, Hasegawa H, Cheng HJ, Little WC and Cheng CP. Endogenous {beta}3-Adrenoceptor Activation Contributes to Left Ventricular and Cardiomyocyte Dysfunction in Heart Failure. *Am J Physiol Heart Circ Physiol* .: 2004.

Prescott, Ed., Methods in Cell Biology, Volume XIV, Academic Press, New York, N.Y. (1976).

Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pa.

Souza, J., *et al.*, *Curr. Pharm. Des.* 2001, 7:1433.

The Merck Index, (13th Edition), Merck & Co., Whitehouse Station, N.J., USA, 2004.

Vakili BA, Okin PM, Devereux RB. Prognostic implications of left ventricular hypertrophy. *Am Heart J.* 2001 Mar;141(3):334-41.

Verdecchia P, Angeli F, Borgioni C, Gattobigio R, de Simone G, Devereux RB, Porcellati, C. Changes in cardiovascular risk by reduction of left ventricular mass in hypertension: a meta-analysis. 2003, *Am J Hypertens.* 2003 Nov;16(11 Pt 1):895-9.

Weber, A. *Annu. Rep. Med. Chem.* 1998, 33:193.

Weyer, C., *et al.*, *Diabetes Metab.* 1999, 25:11.

William, M., Vein, J., and Rasmussen, H. Biphasic effect of atrial natriuretic peptide on the sarcolemmal sodium pump. *Biophys.J. Suppl* 84-2, 191a. 2003.

Yanagisawa T, Sato T, Yamada H, Sukegawa J, Nunoki K. Selectivity and potency of agonists for the three subtypes of cloned human beta-adrenoceptors expressed in Chinese hamster ovary cells. *Tohoku J Exp Med.* 2000 Nov;192(3):181-93.